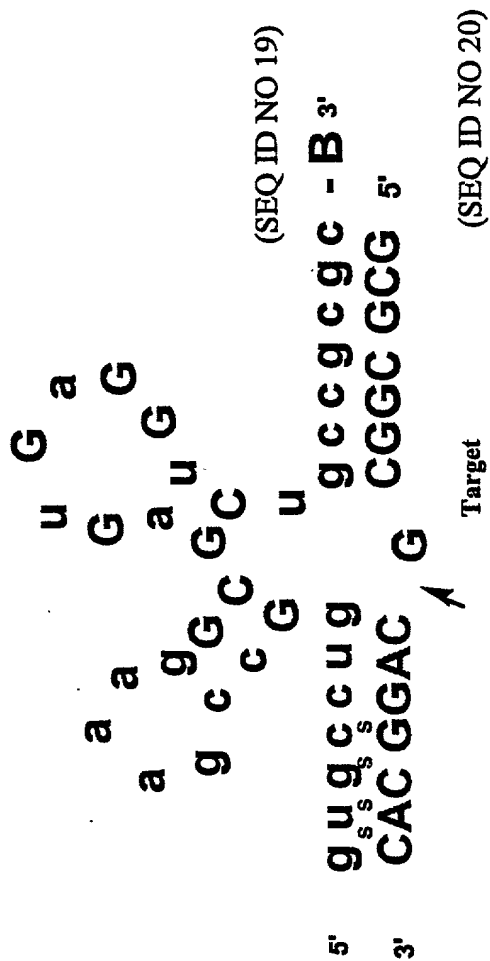


Zinzyne A-motif RZ



Uppercase indicates natural ribo residues

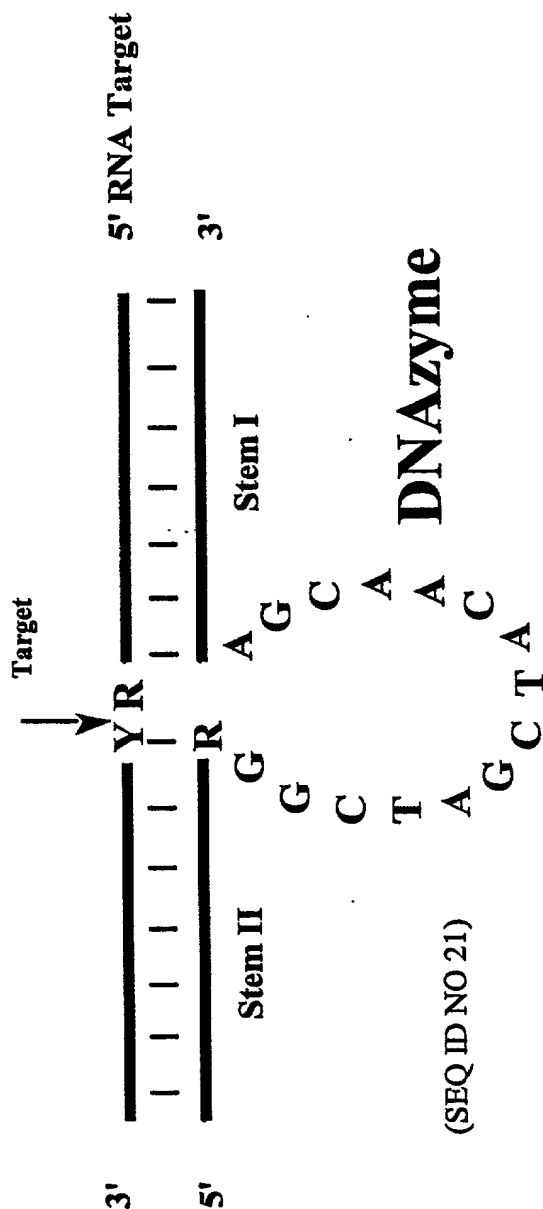
C indicates 2' - d-NH₂-C

Lowercase: 2'-O-Me

Subscript s indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 4: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence

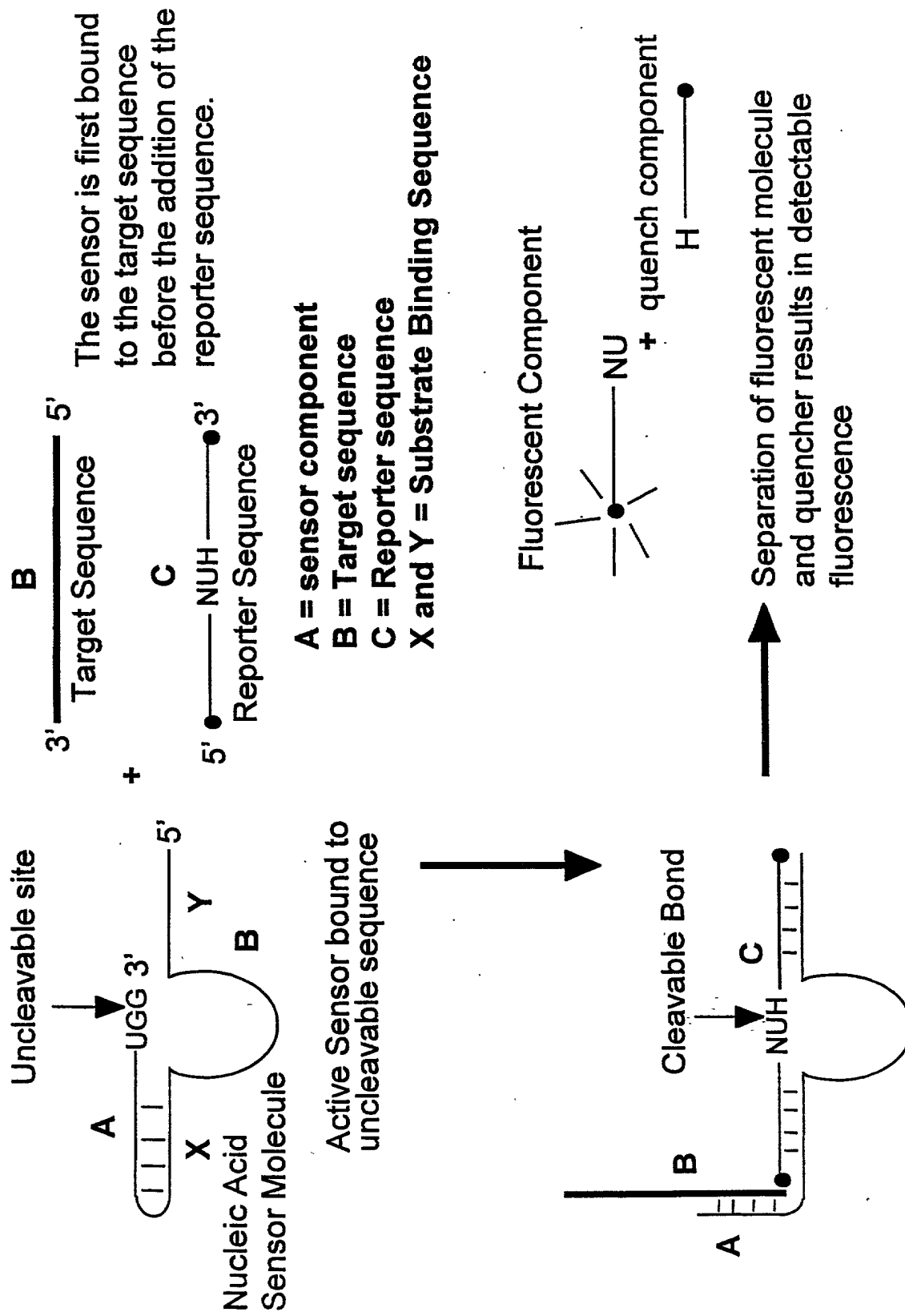


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

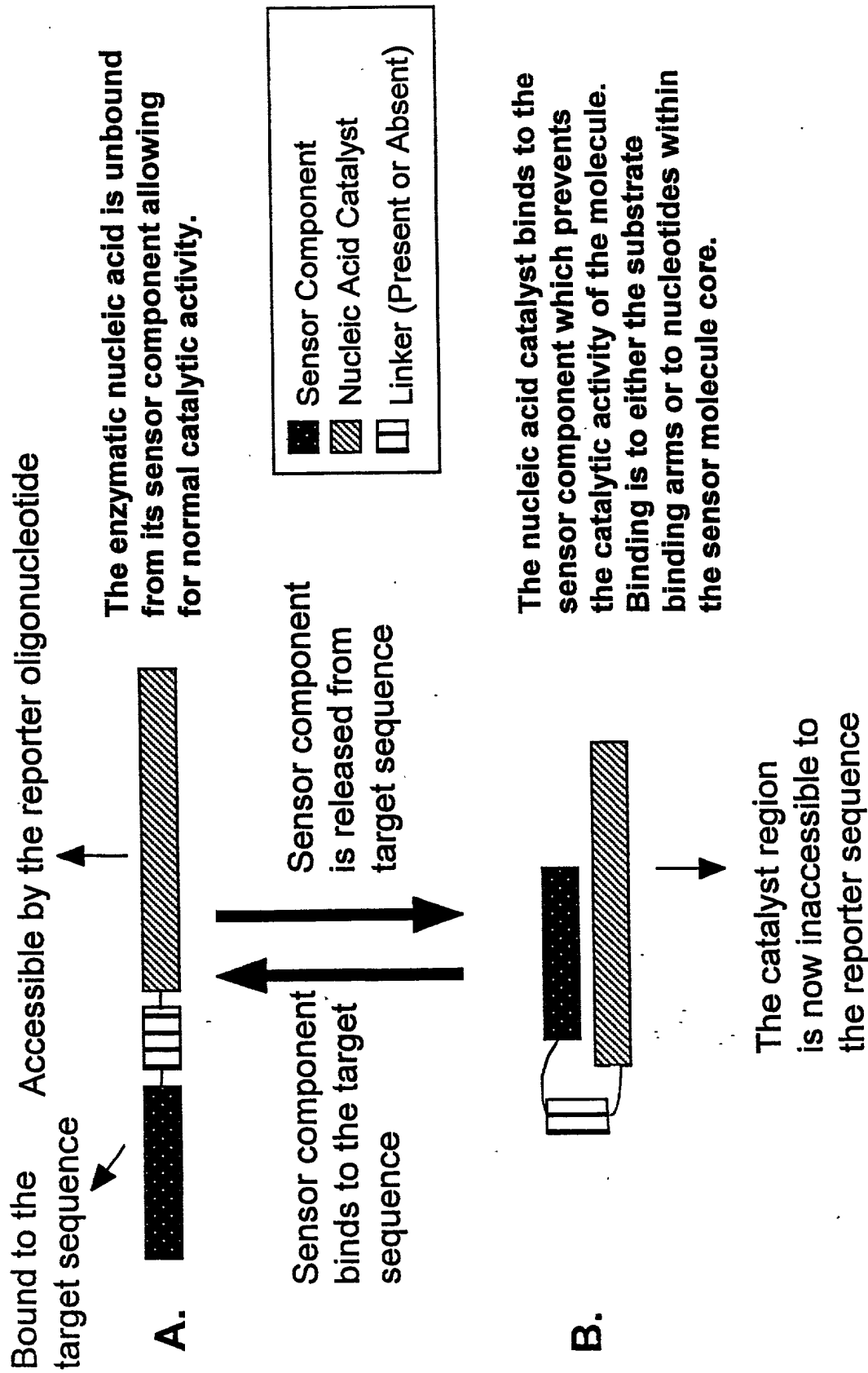


Figure 7. Examples of Nucleic Acid Sensor Molecules

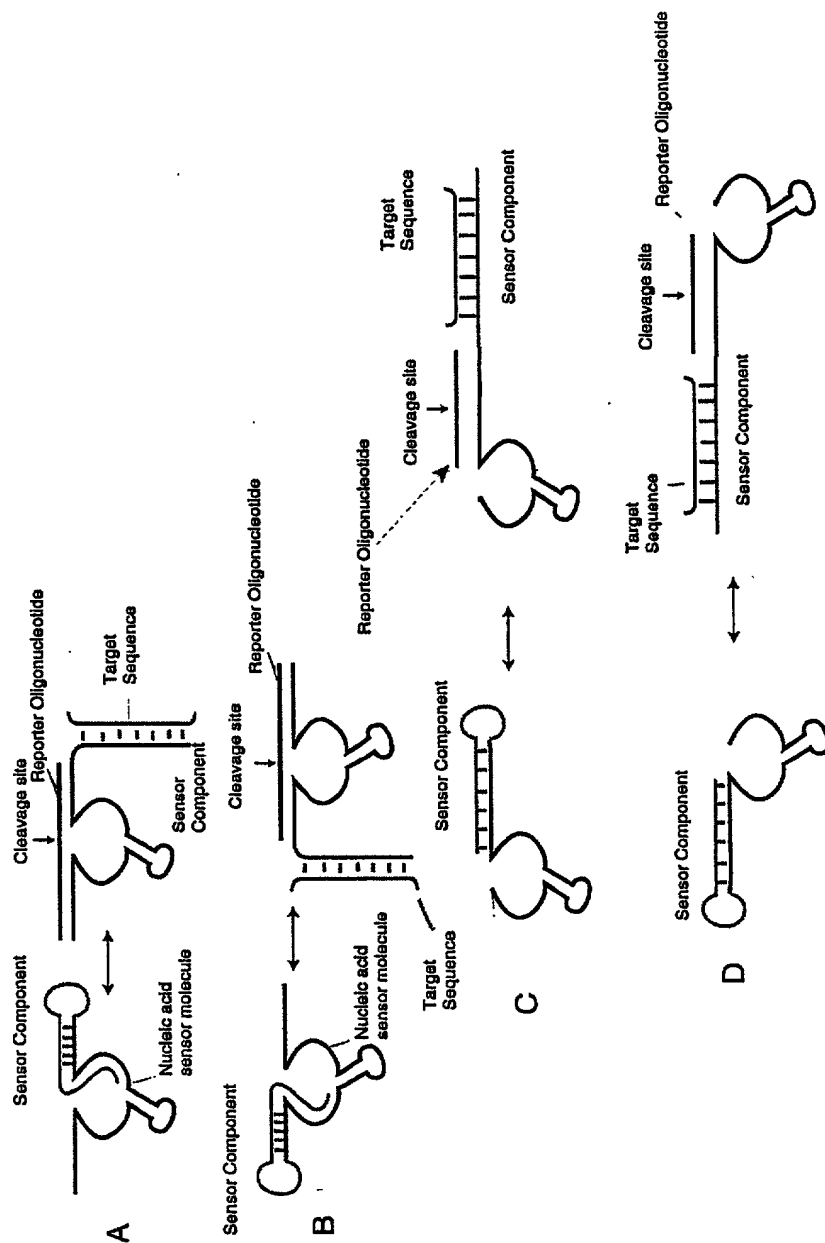


Figure 8. Examples of Nucleic Acid Sensor Molecules

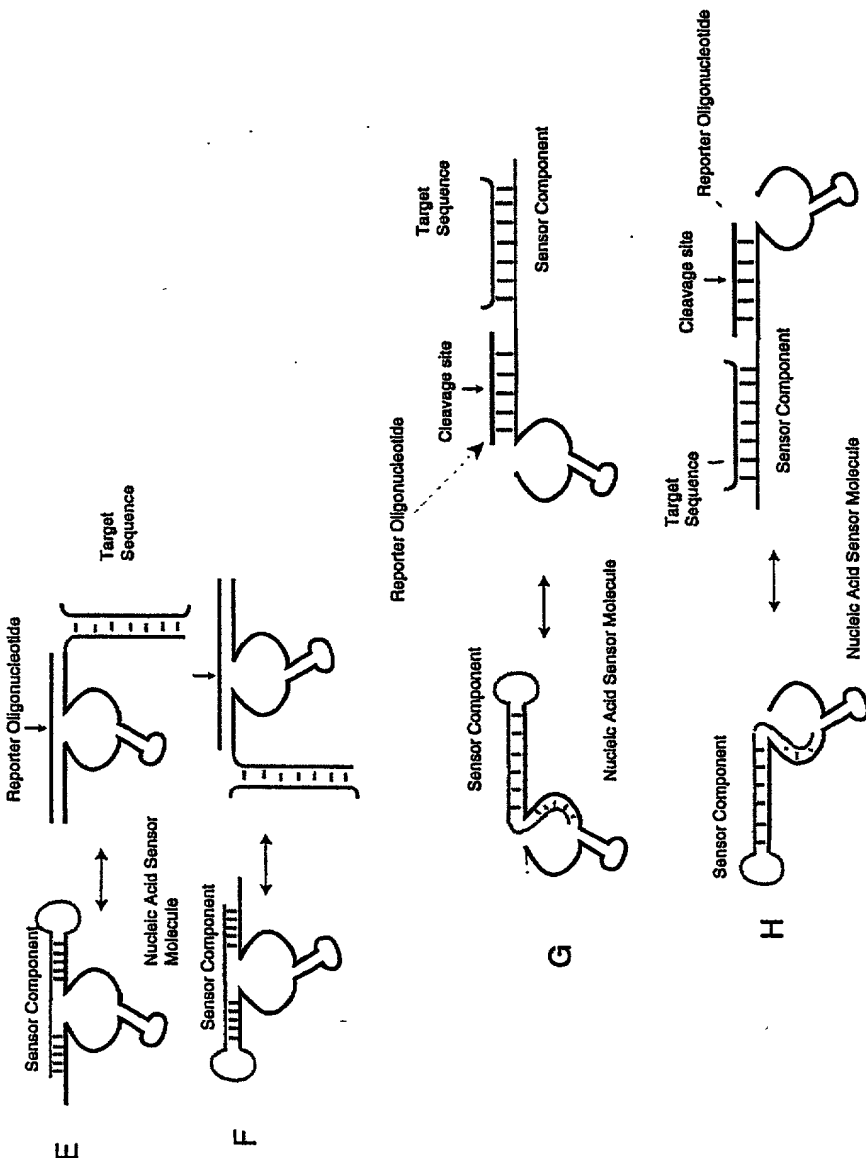


Figure 9. Examples of Nucleic Acid Sensor Molecules

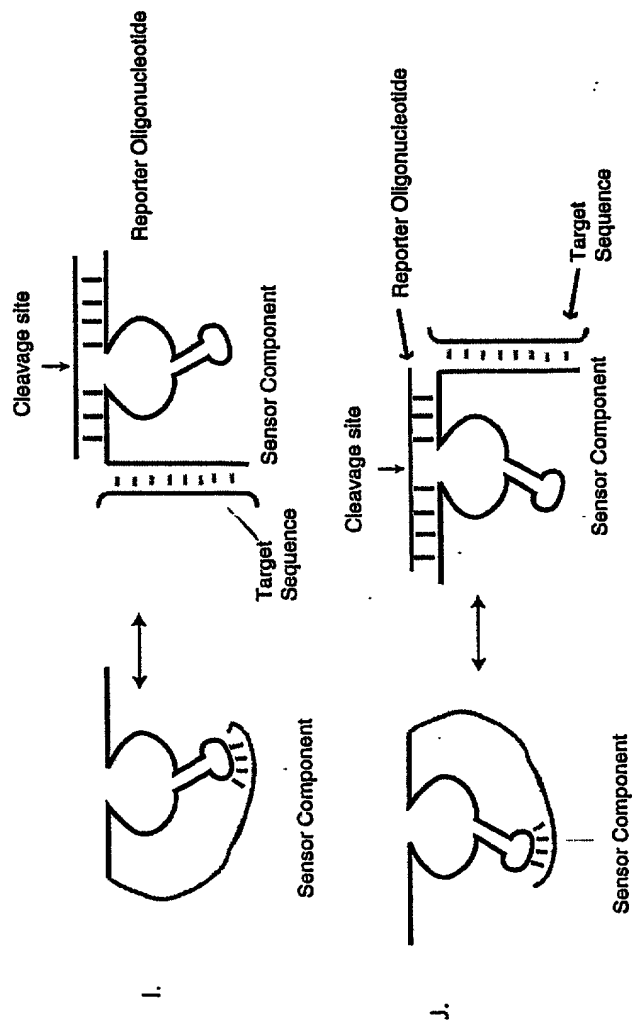
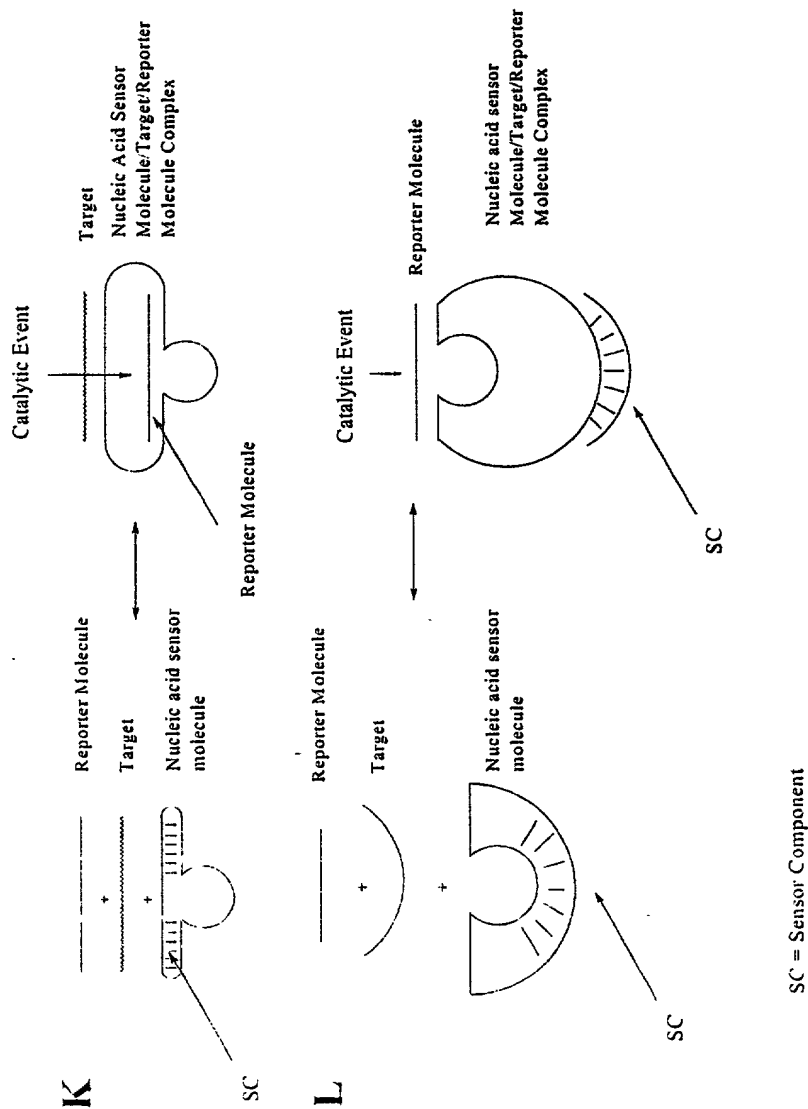


Figure 10: Examples of Nucleic Acid Sensor Molecules



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Figure 11: Examples of Nucleic Acid Sensor Molecules

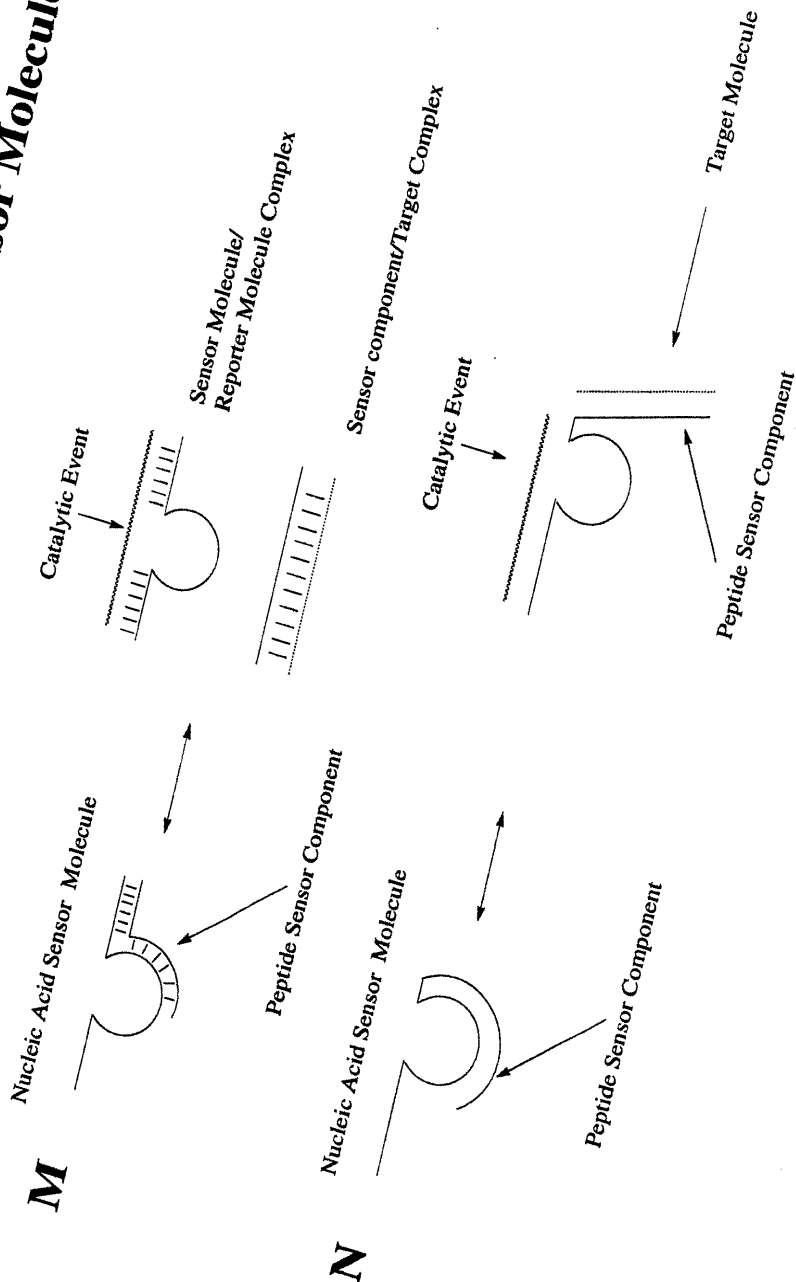
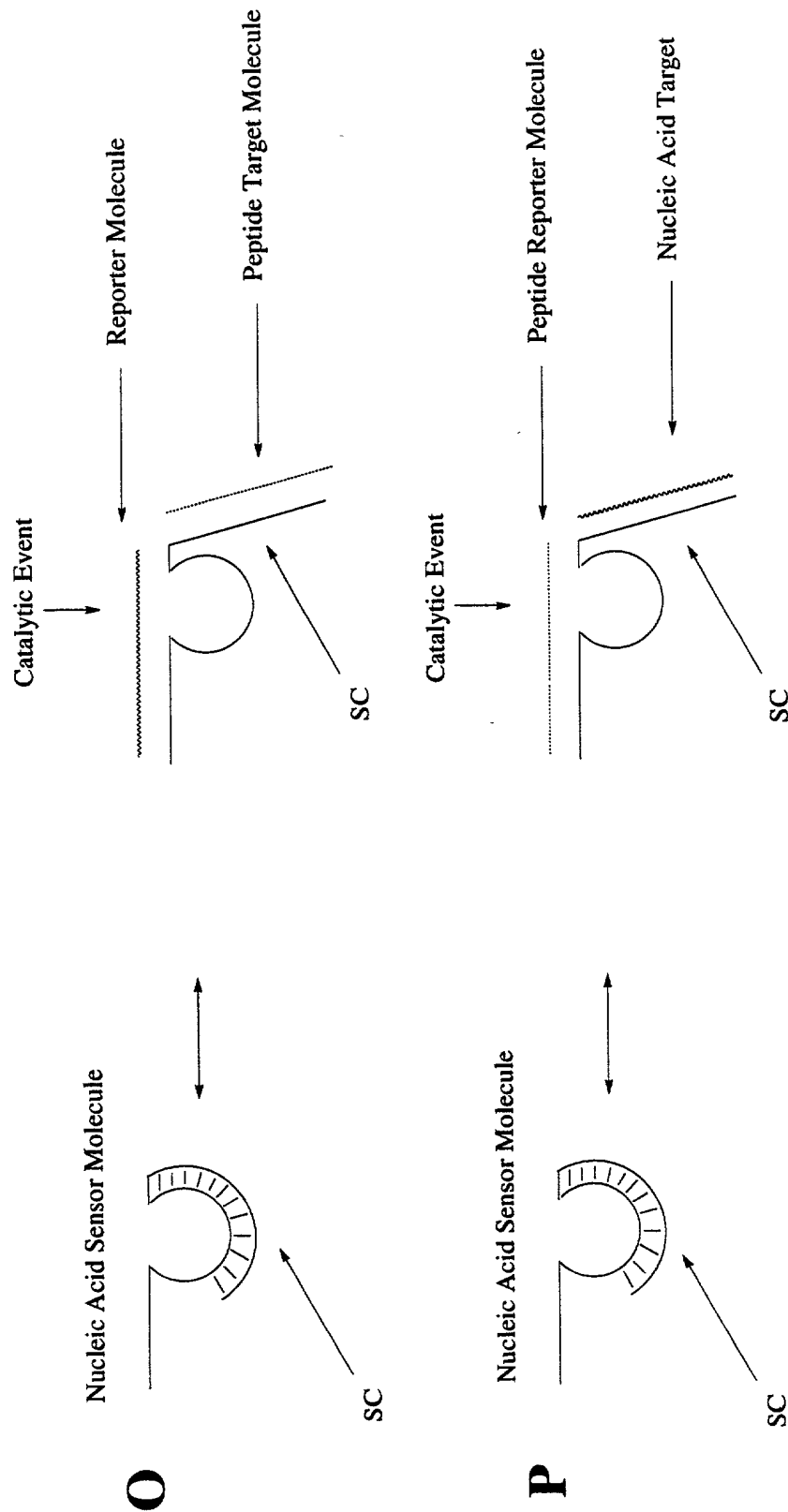


Figure 12: Examples of Nucleic Acid Sensor Molecules



SC = Sensor Component

Figure 13: Examples of Nucleic Acid Sensor Molecules

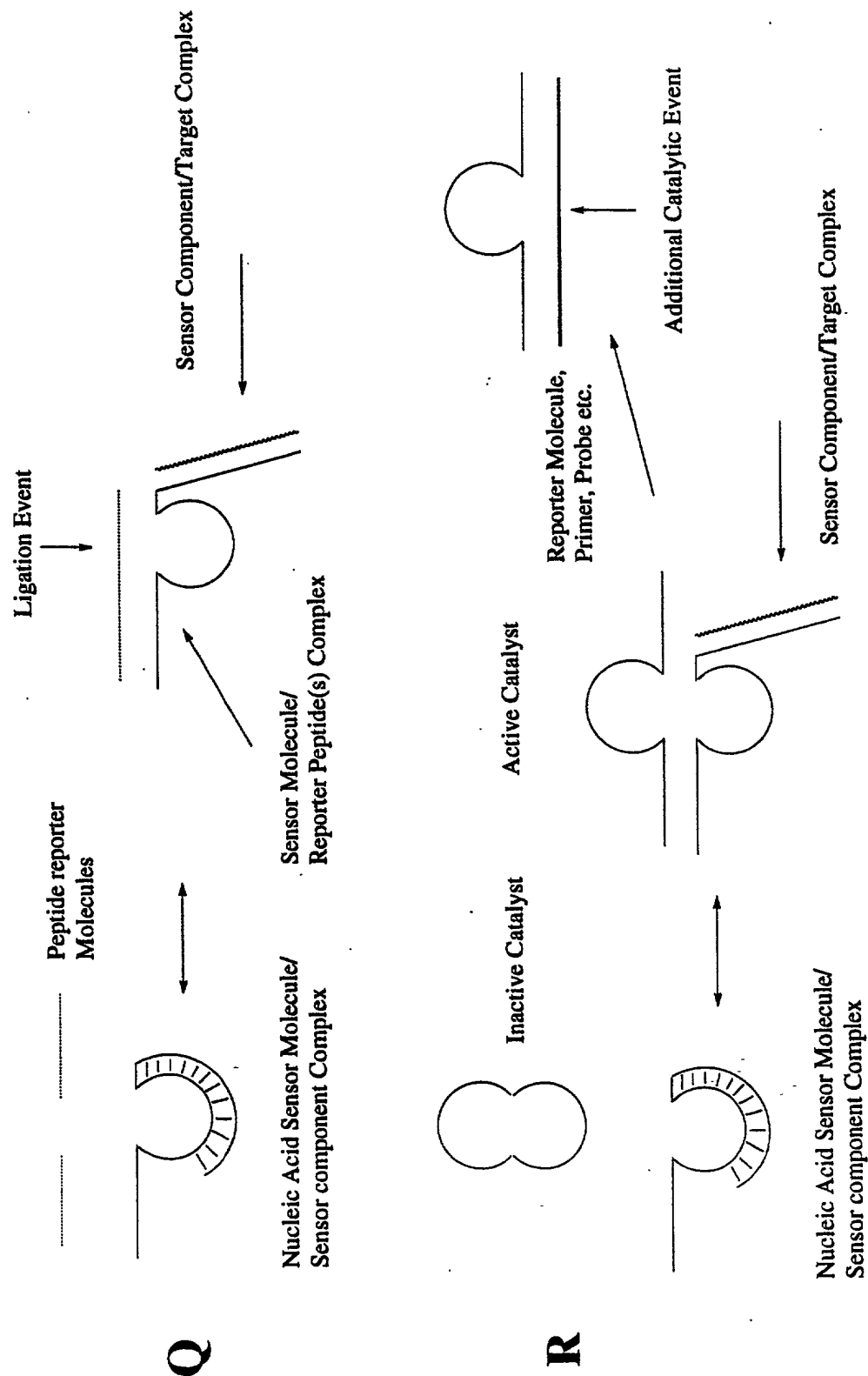


Figure 14: Inherent Amplification of Signal

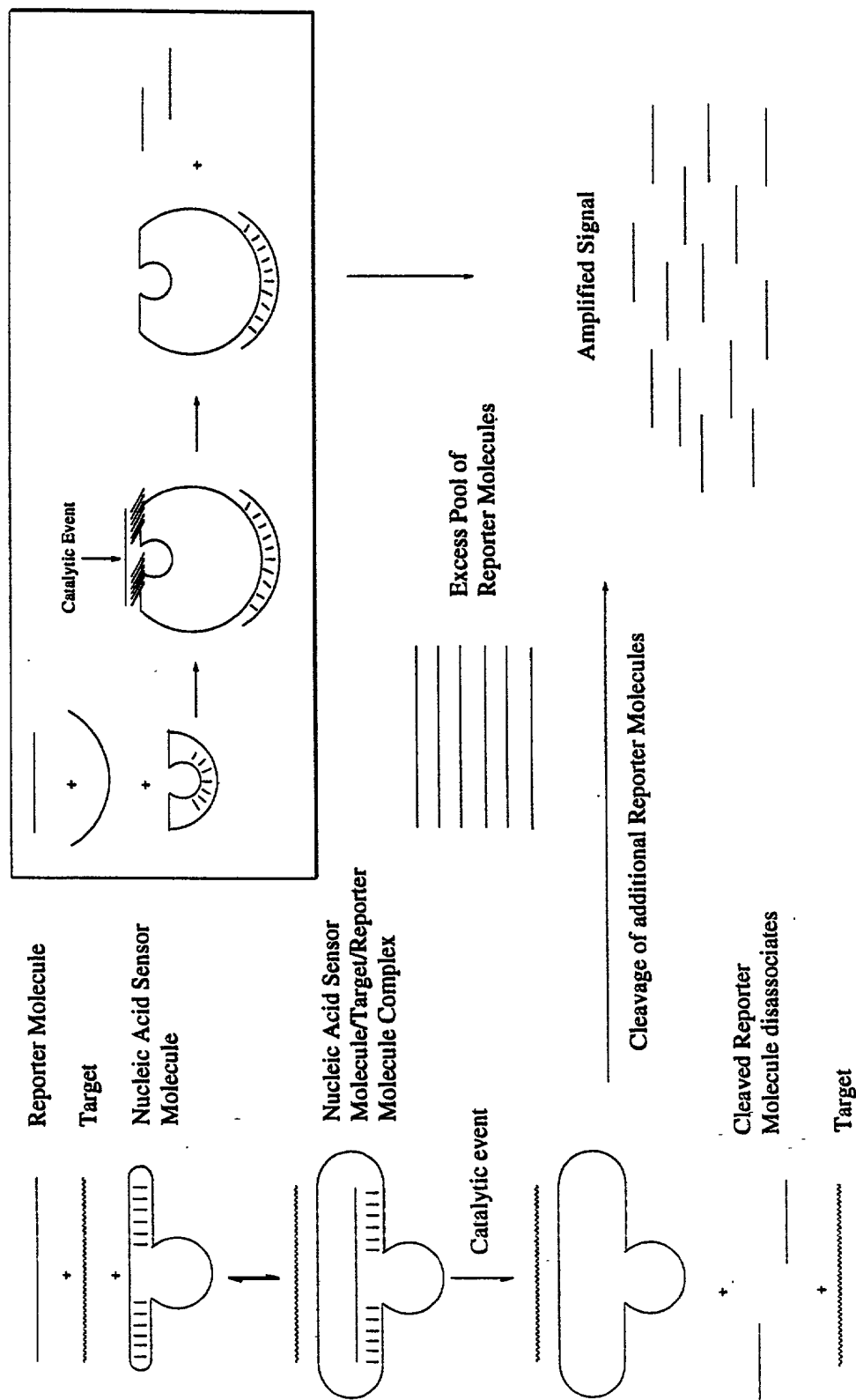


Figure 15: Example of Diagnostic System

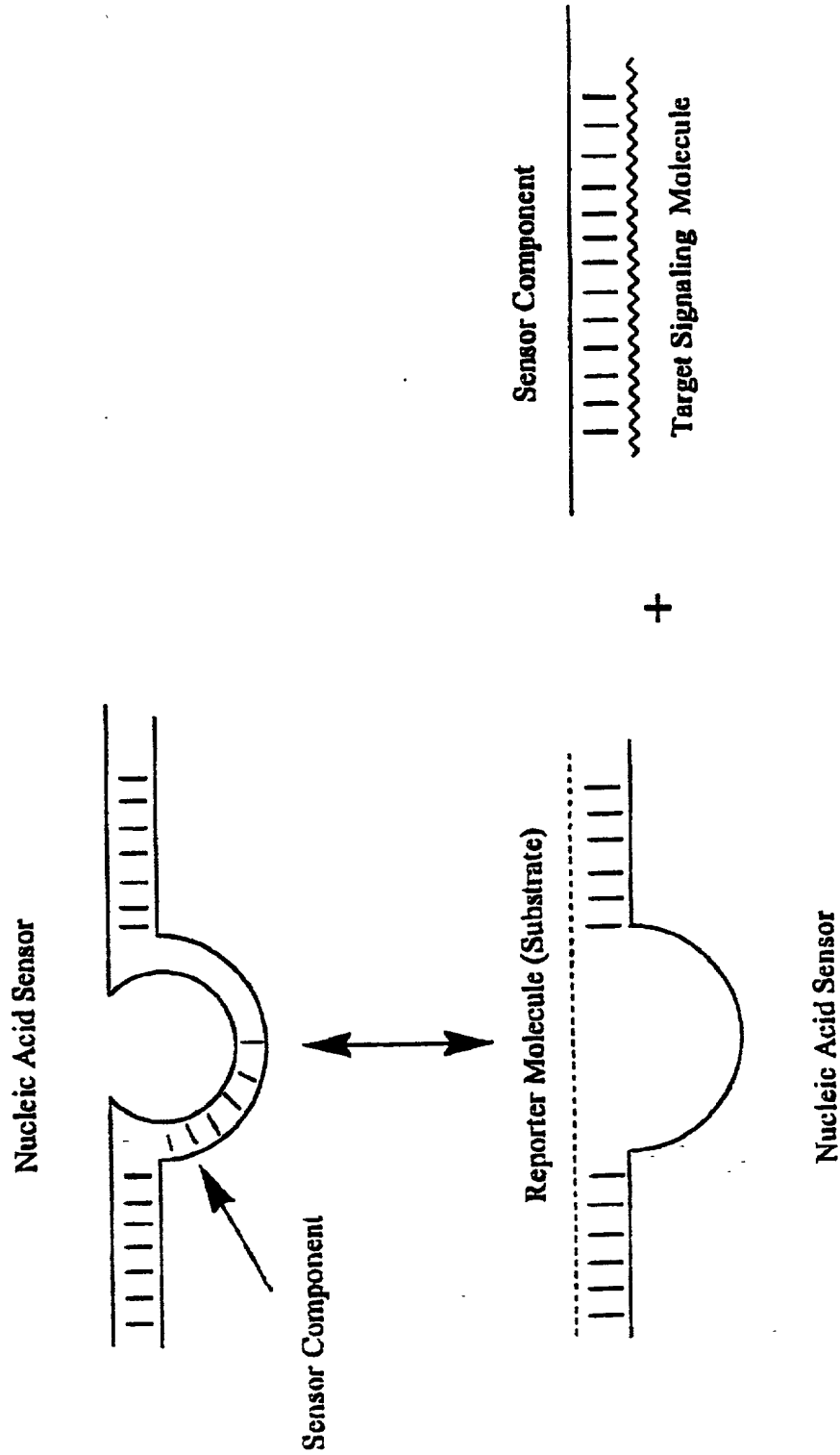
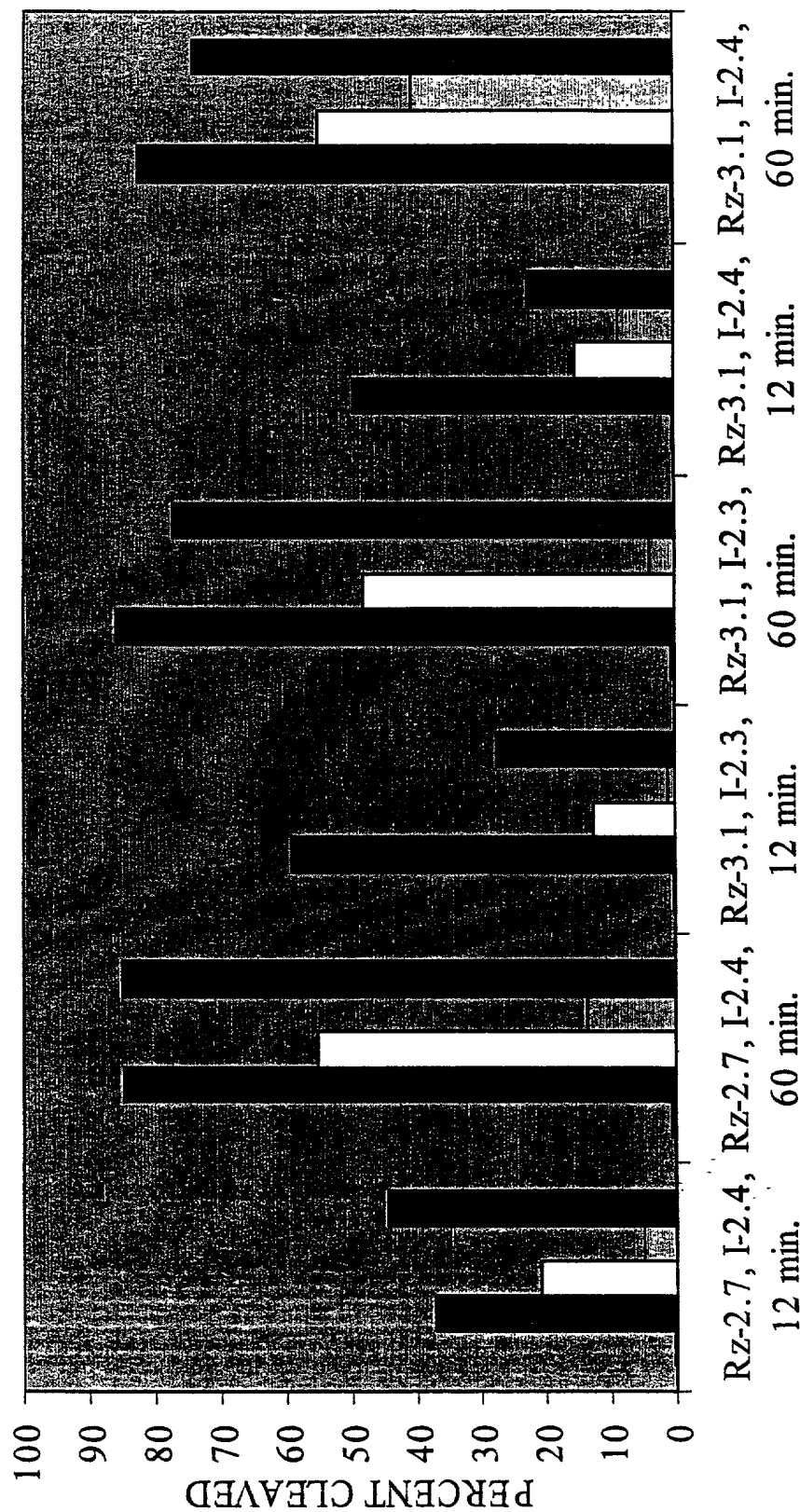


Figure 16: Ribozyme Diagnostic Screen

INHIBITORY FOLDING WITH TARGET RESCUE



No Rz
 +Rz @ 10 nM
 +Rz @ 20 nM
 +Rz @ 200 nM
 +Rz @ 500 nM

Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

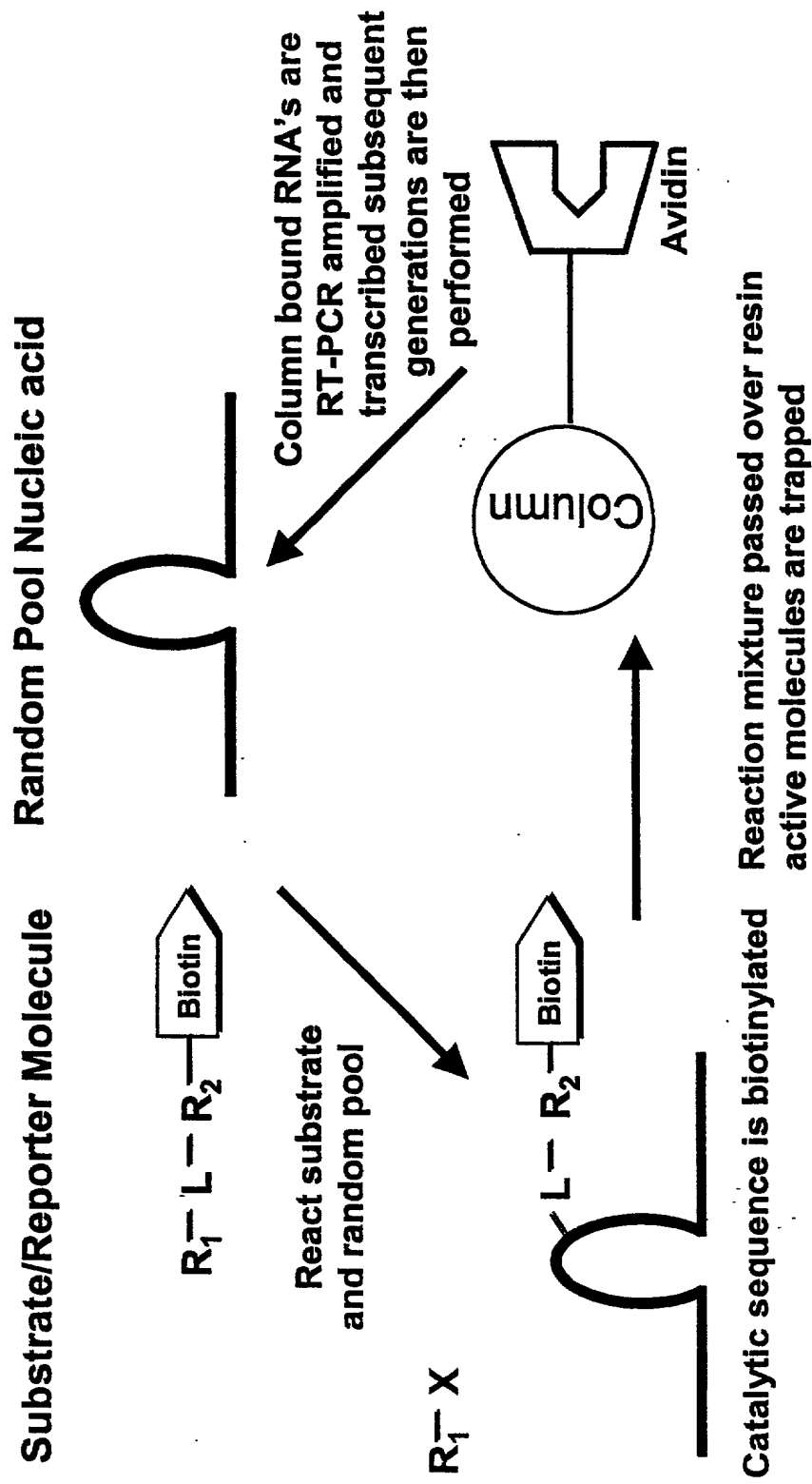


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

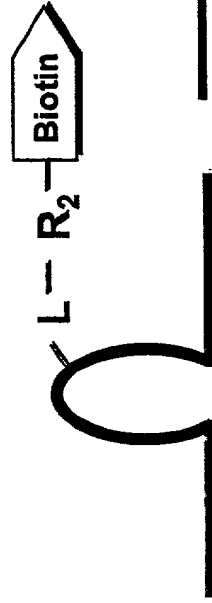
Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
- + Ligand (second round)



React substrate
and random pool

$R_1 - X$



Scheme I

Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.

Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

Catalytic sequence is biotinylated

Reaction mixture passed over resin
active molecules are trapped

Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules - Ligand dependent



Scheme II

- Perform an entire selection as shown in first slide (in the absence of the Ligand)
- Mutagenize the winning pool
- Perform an entire selection using this pool with the requirement of Ligand binding.
- Mutagenize this pool
- Repeat original selection (for activity) in the presence of Ligand - countselect for molecules that react in the absence of ligand

Catalytic sequence is biotinylated

Reaction mixture passed over resin
active molecules are trapped

Figure 18a: Isomerase Nucleic Acid Sensor Molecule – Selection Scheme

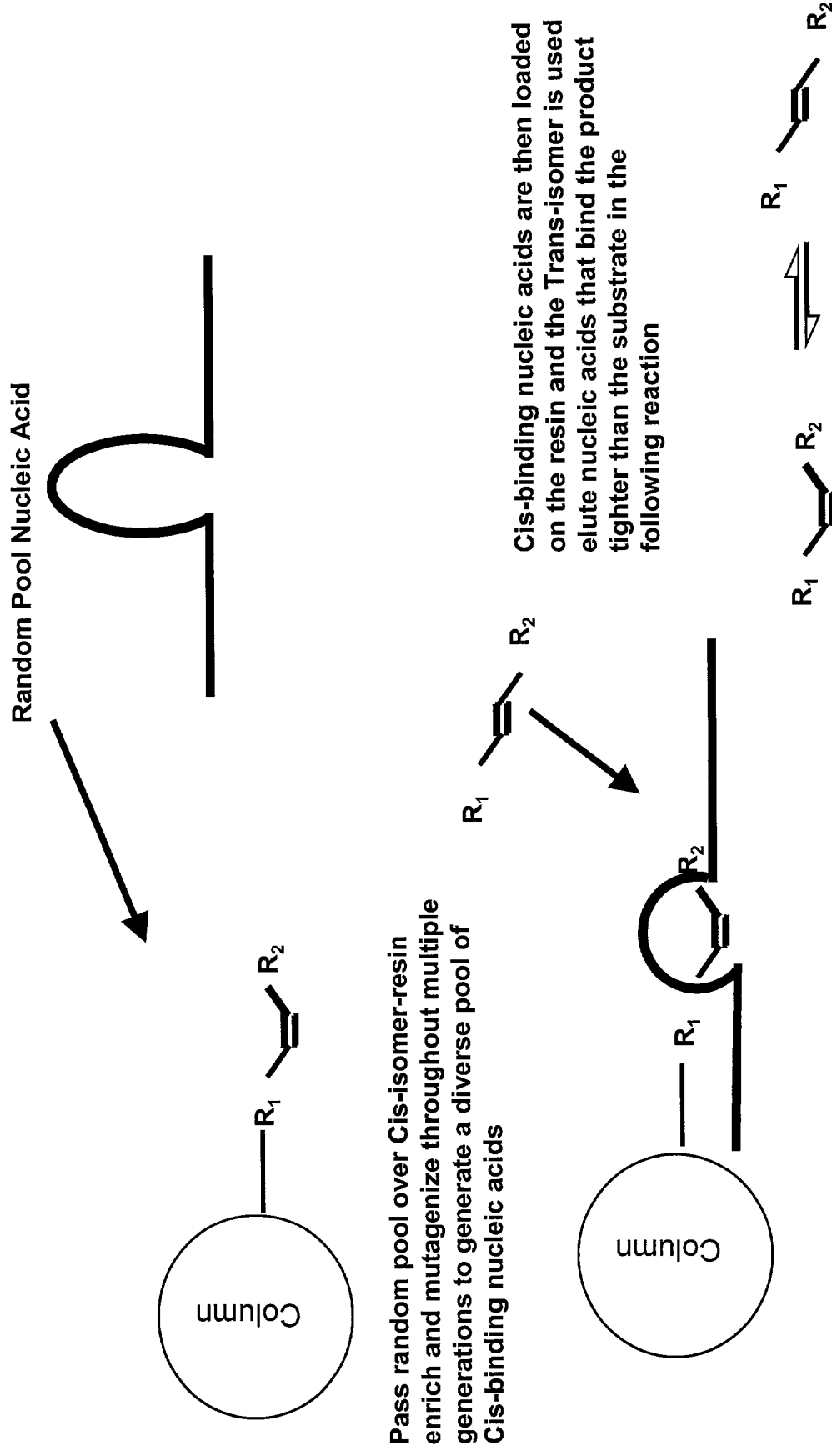
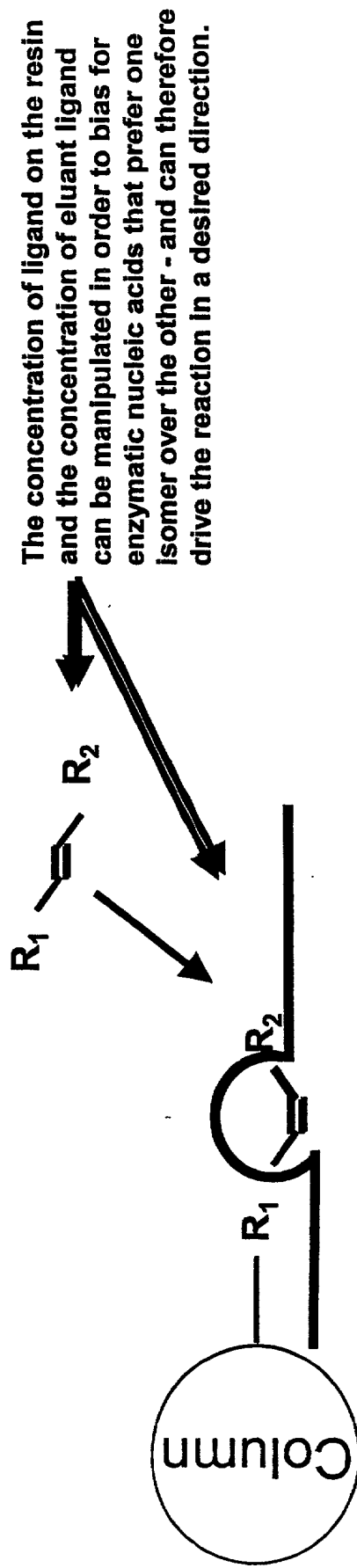


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme

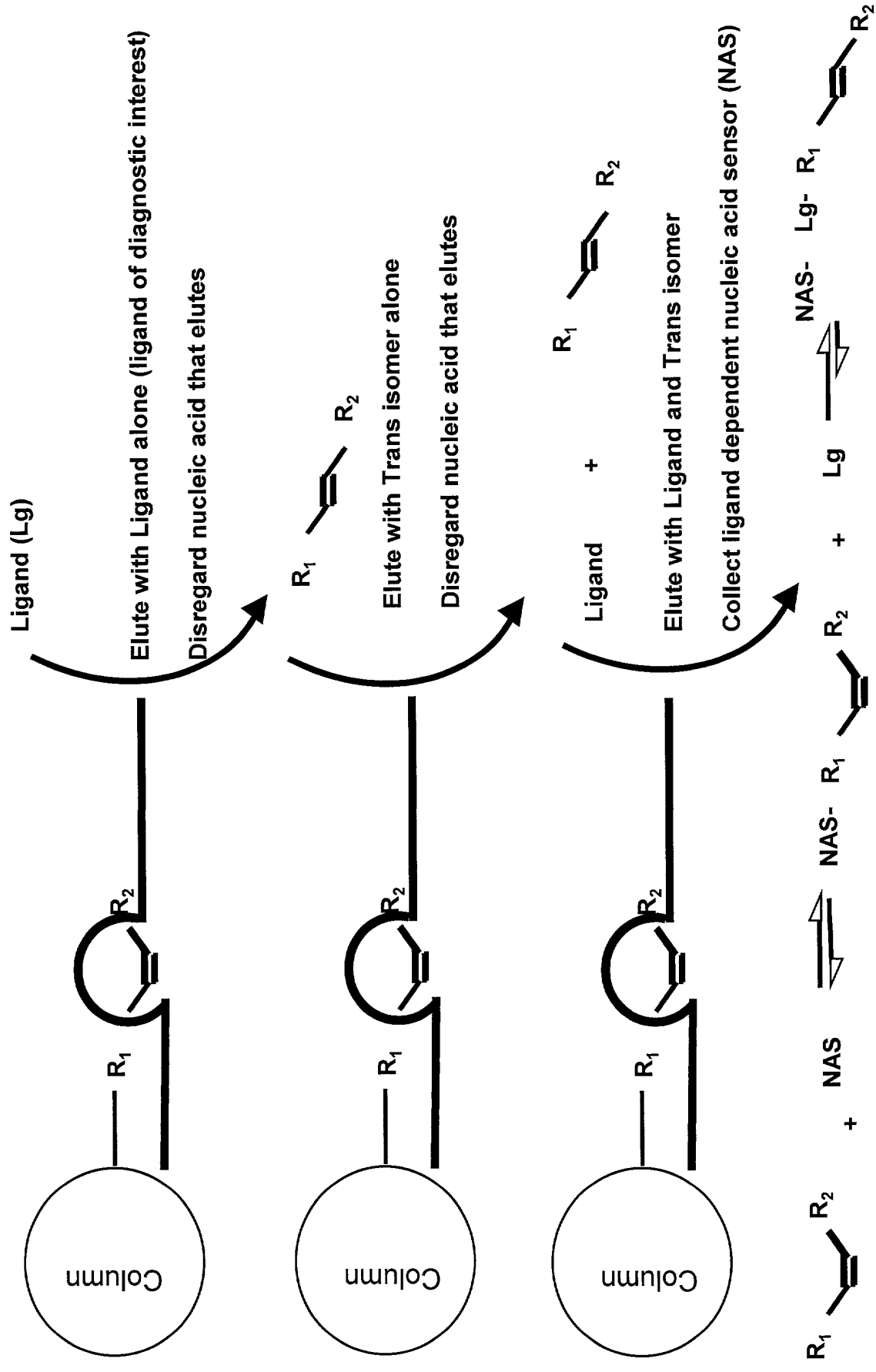


E.g. Selection for Cis-Isomer at 100 μM - yield $\text{cis}K_d = 100 \mu\text{M}$
 Elute with Trans-isomer at 0.1 μM - yield $\text{trans}K_d = 0.1 \mu\text{M}$

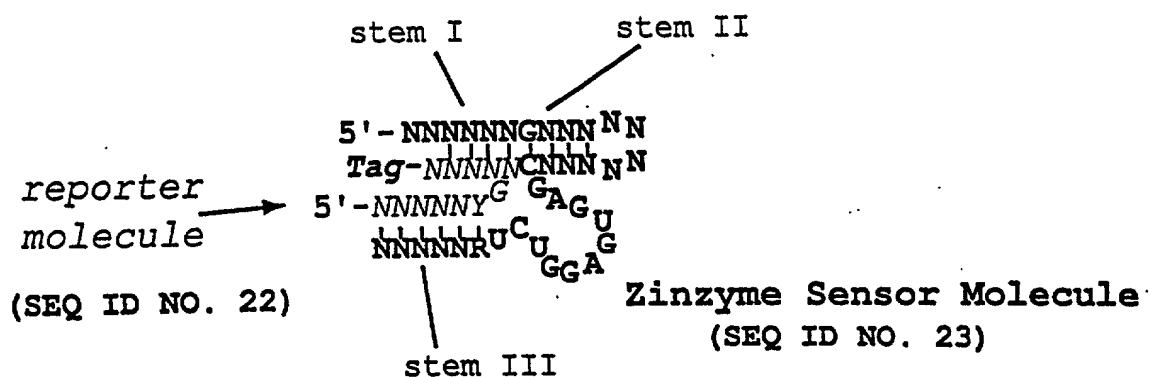
Isolate catalysts for the reaction below



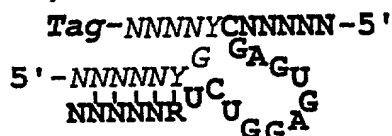
Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent



Zinzyme Sensor Molecule for detection of Nucleic Acid



Inactive Zinzyme sensor/
reporter molecule complex
(SEQ ID NO. 22)

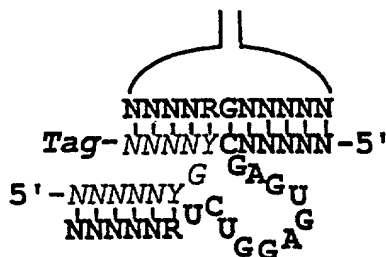


(SEQ ID NO. 24)

Target Signaling
Molecule



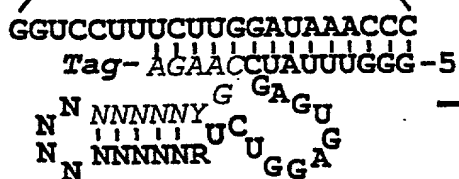
Target Signaling
Molecule
(SEQ ID NO. 25)



Active Zinzyme sensor/
reporter molecule complex

Stem-loop III of HCV (SEQ ID NO. 26)
(SEQ ID NO. 22)

Active HCV Zinzyme sensor/
reporter molecule complex
(SEQ ID NO. 27)

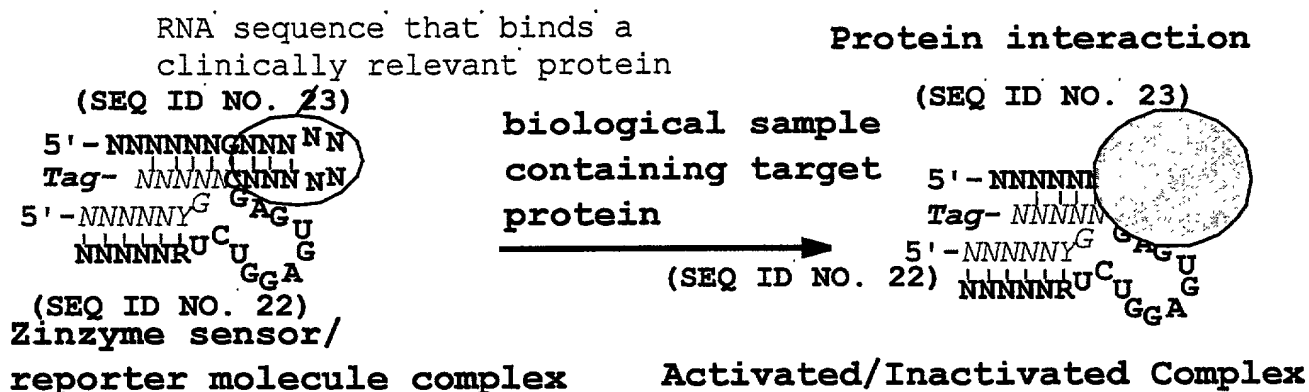
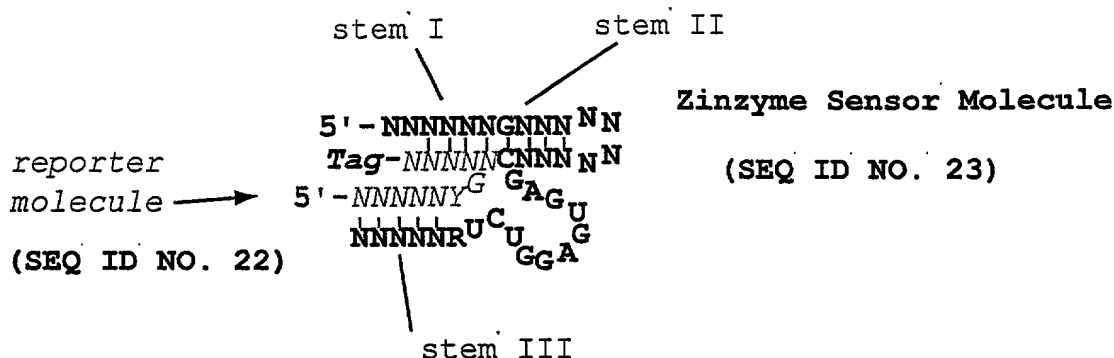


catalysis results in
release of Tag-AGAAC
for detection

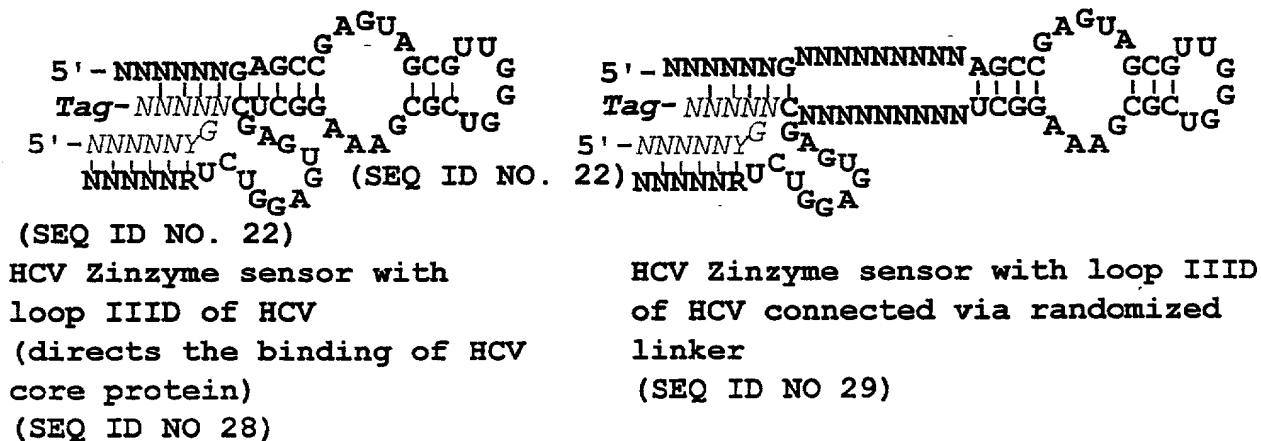
Zinzyme sensor can be attached to solid support/surface,
for example at the 5'-end

FIG. 19

Figure 20: Zinzyme Sensor Molecule for detection of Protein

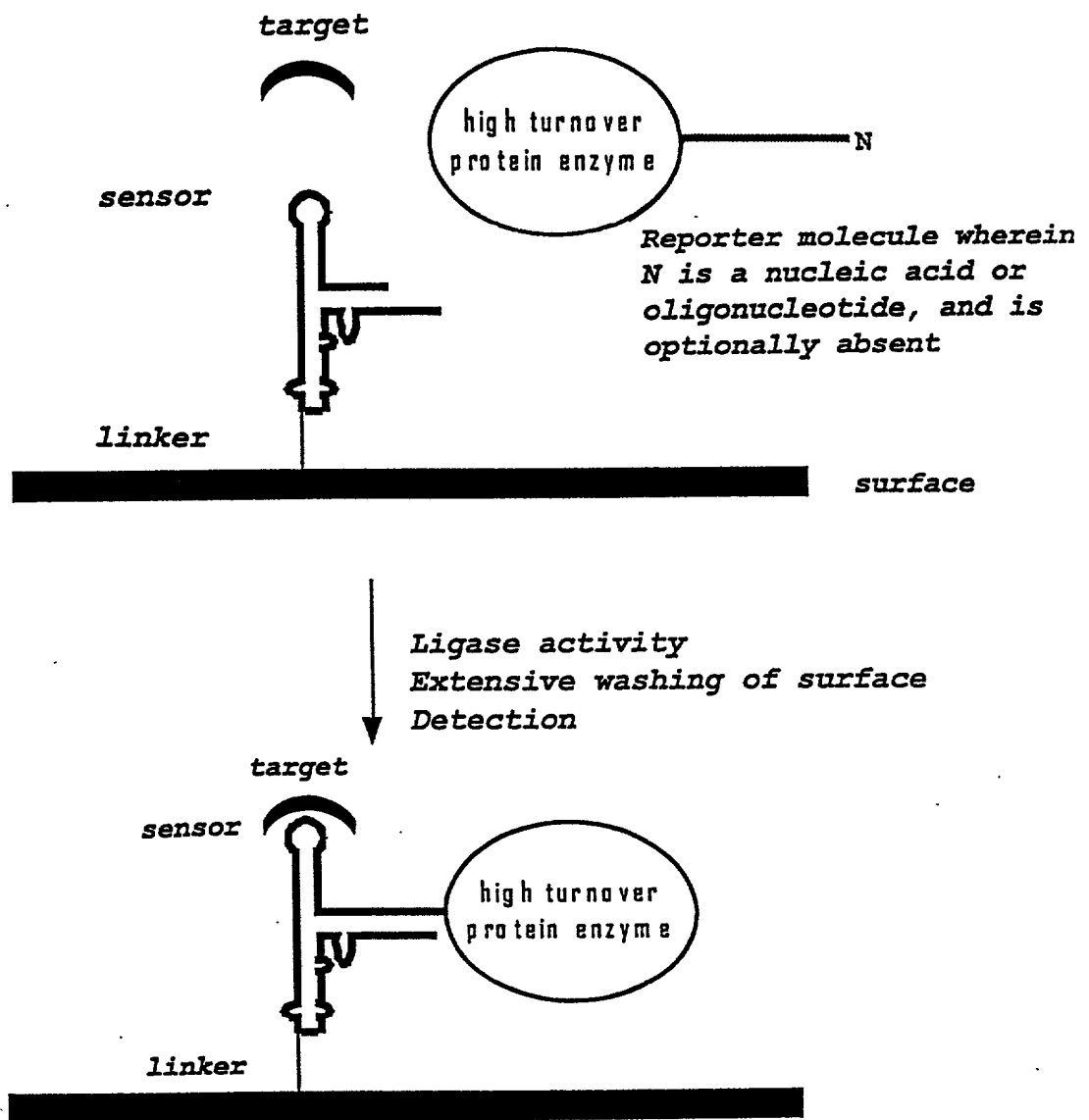


Sensor/reporter complex for detection of HCV core protein



[illegible]

Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

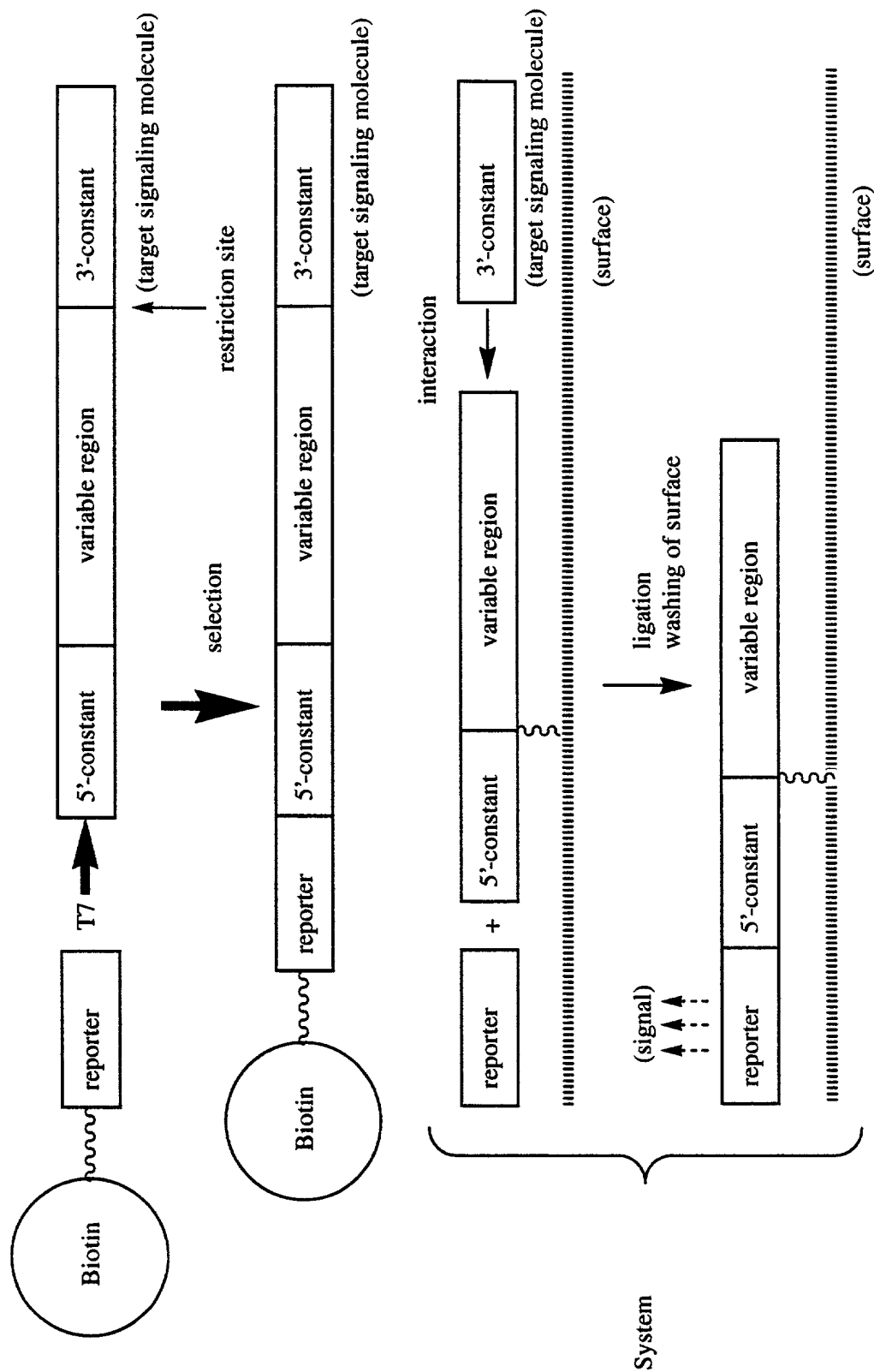


Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit

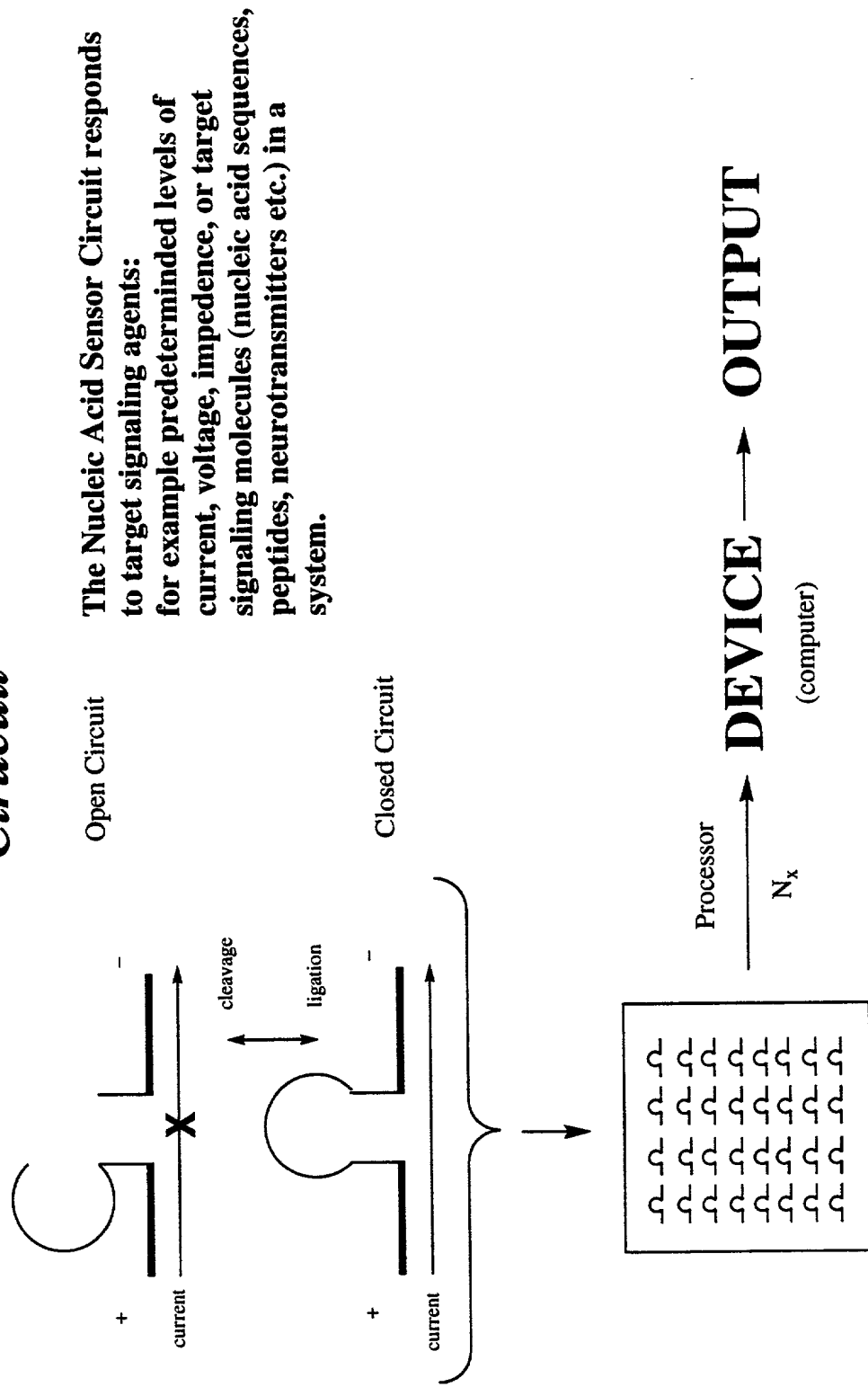
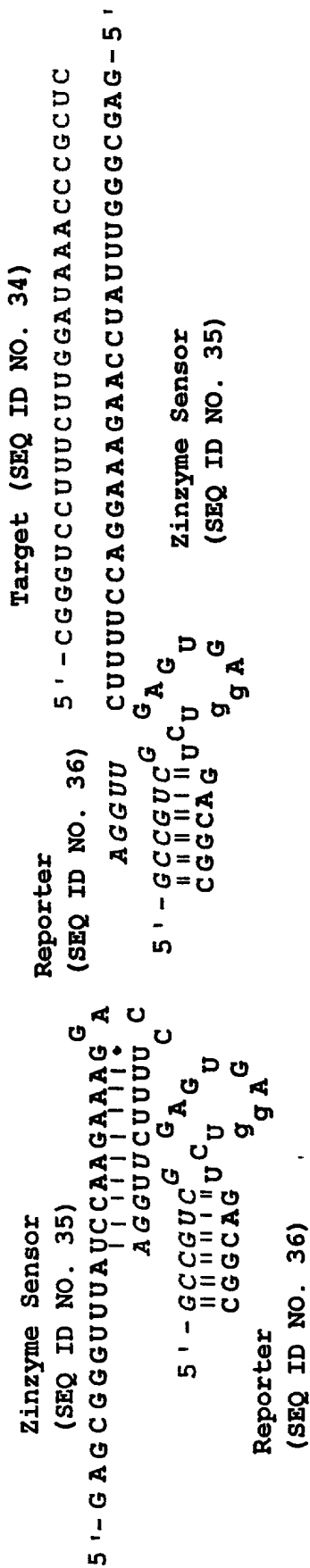


Figure 26: Target Inactivation of Zinzyme Sensor Molecule



ACTIVE \longleftrightarrow TARGET INACTIVATED

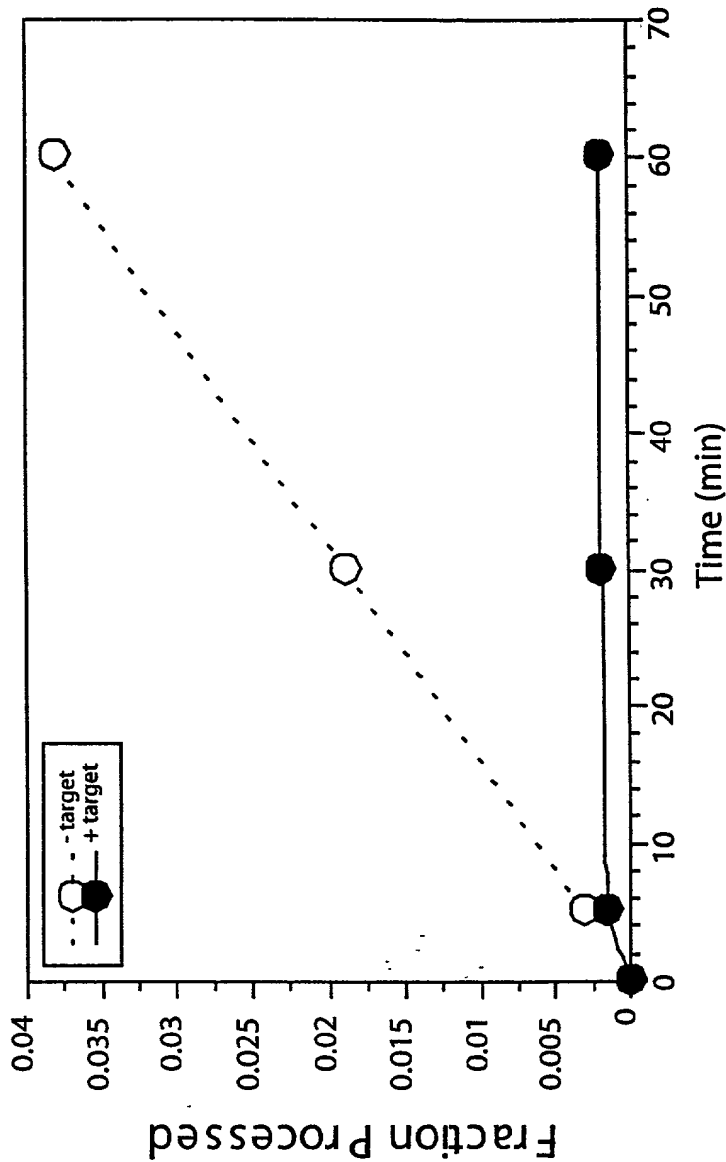
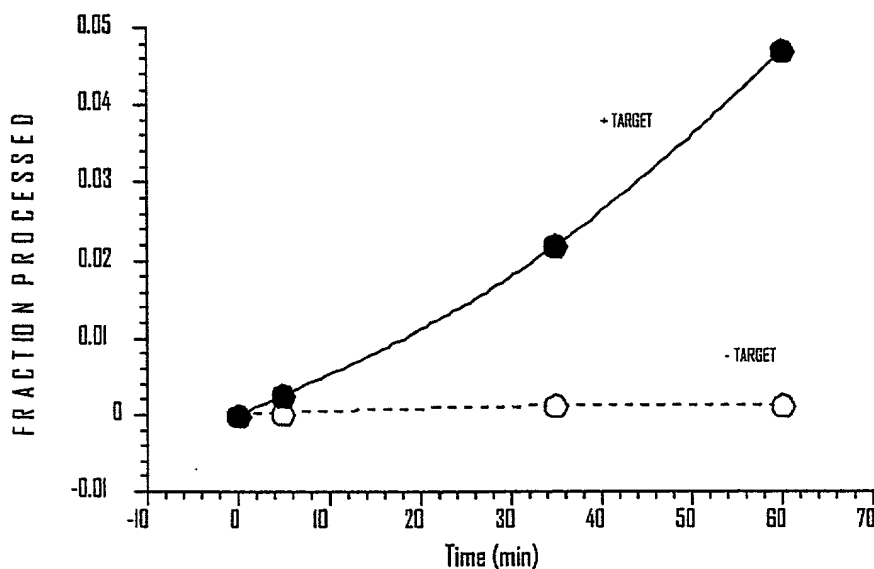
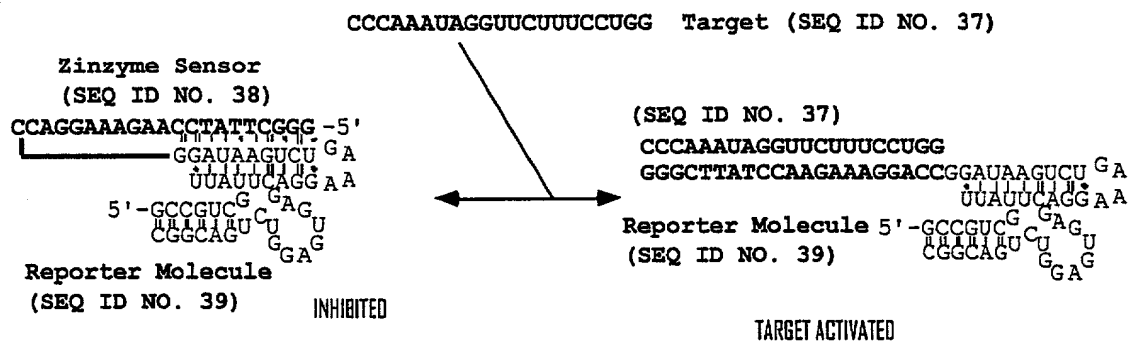


Figure 27: Target Activation of Zinzyme Sensor Molecule



09877526-100201

Figure 28: Erk modulated Nucleic Acid Sensor Molecule

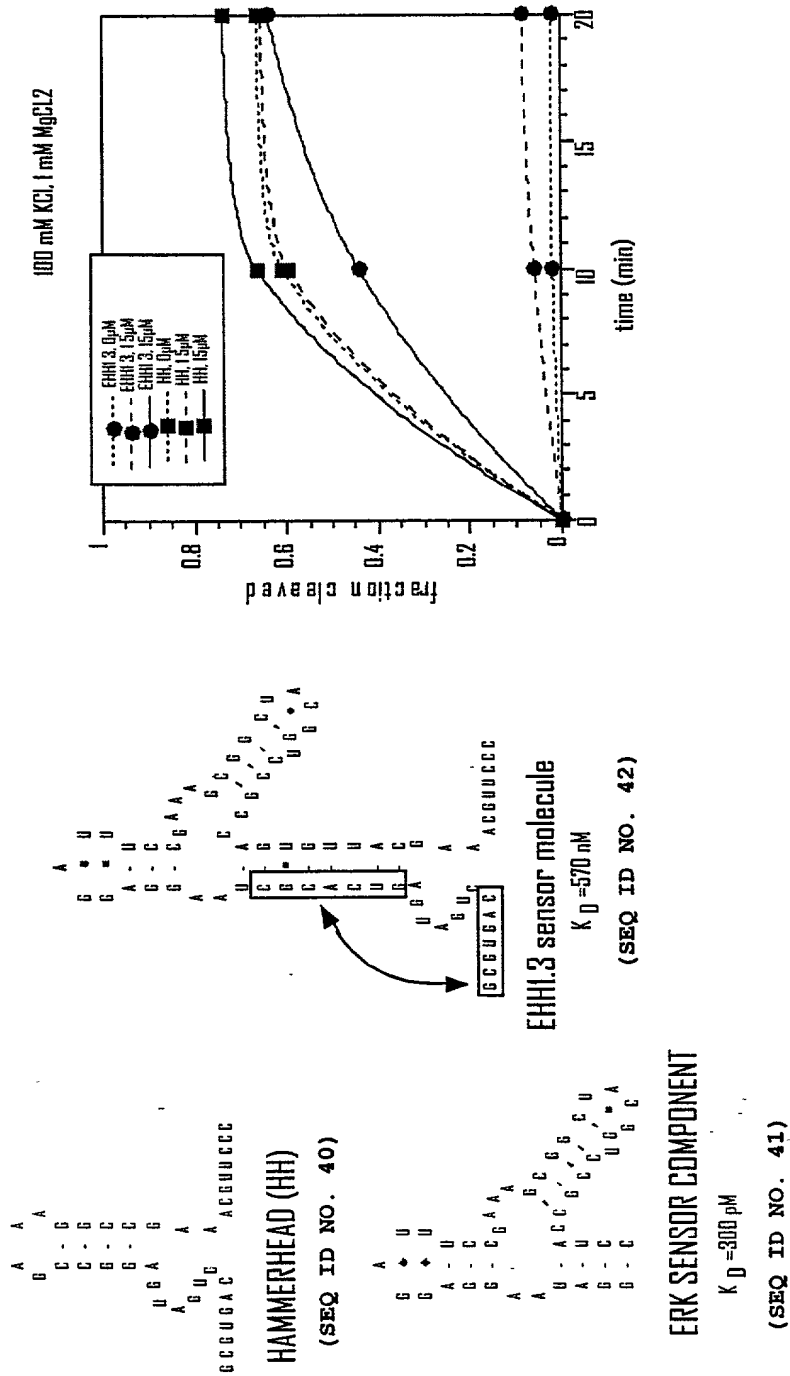


Figure 30

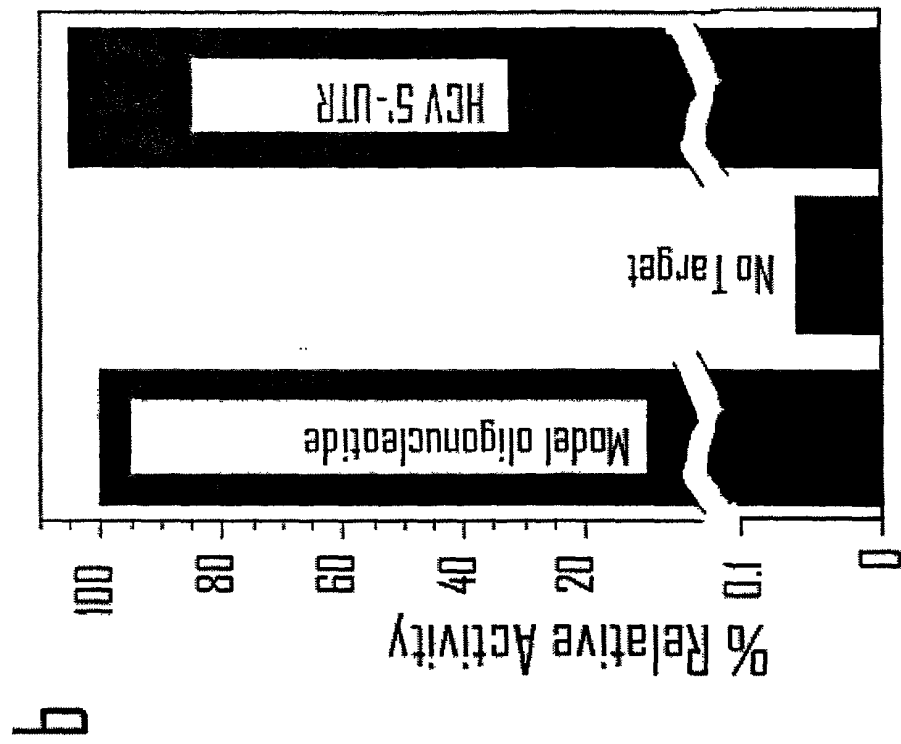
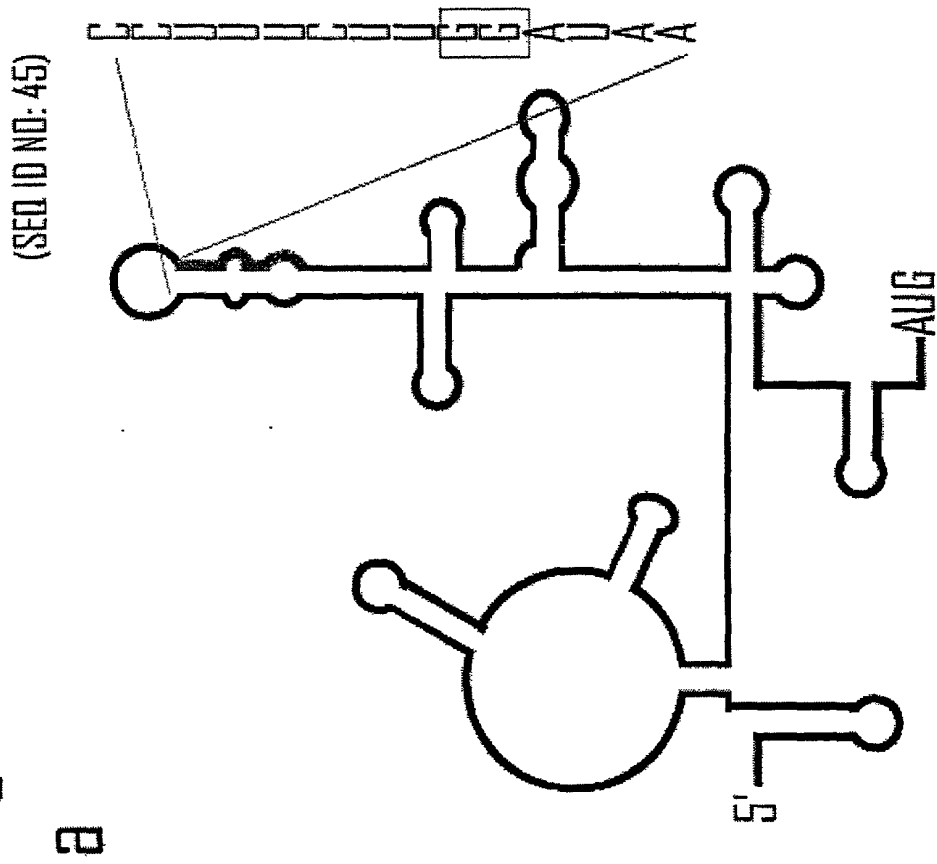


Figure 31

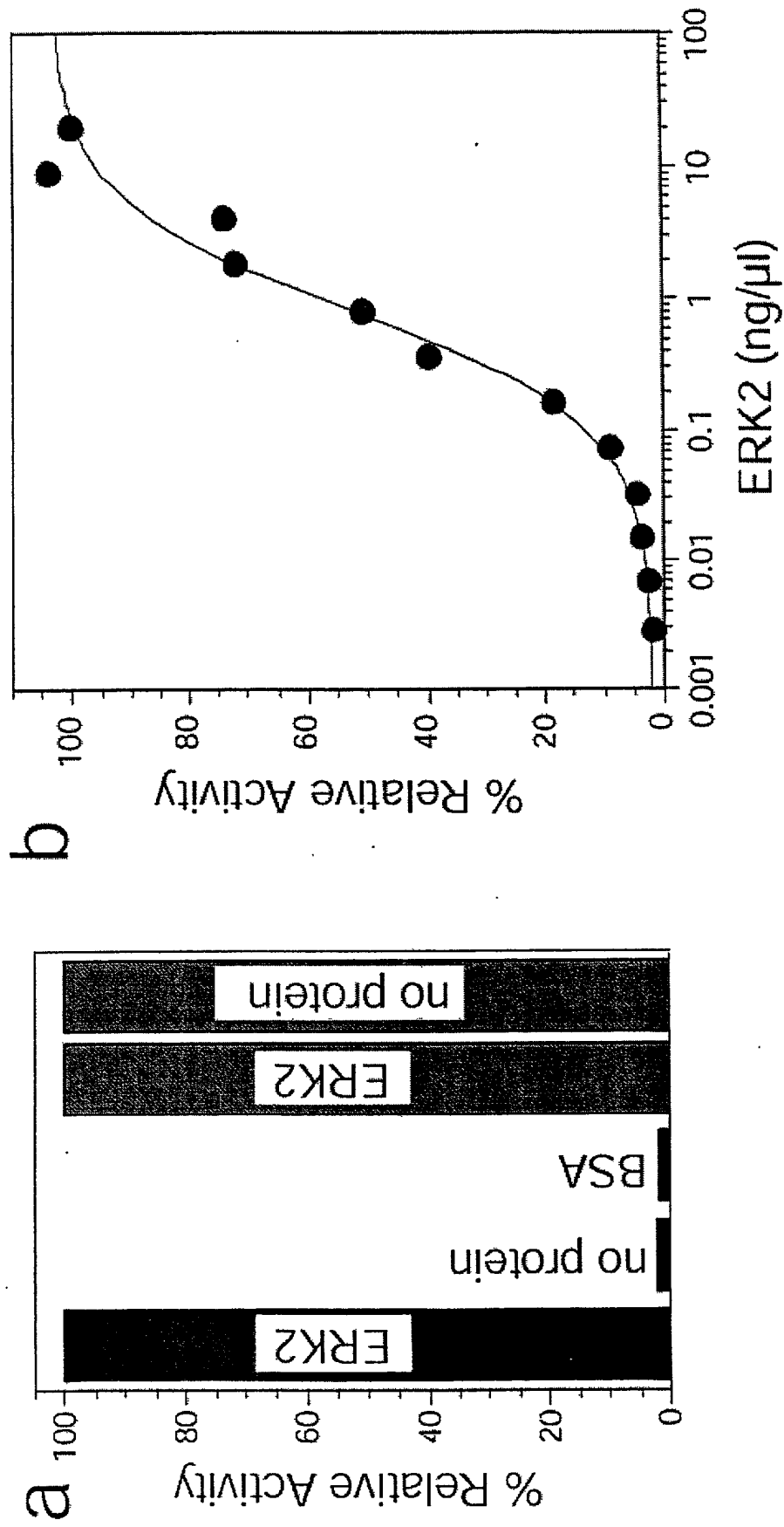


Figure 32

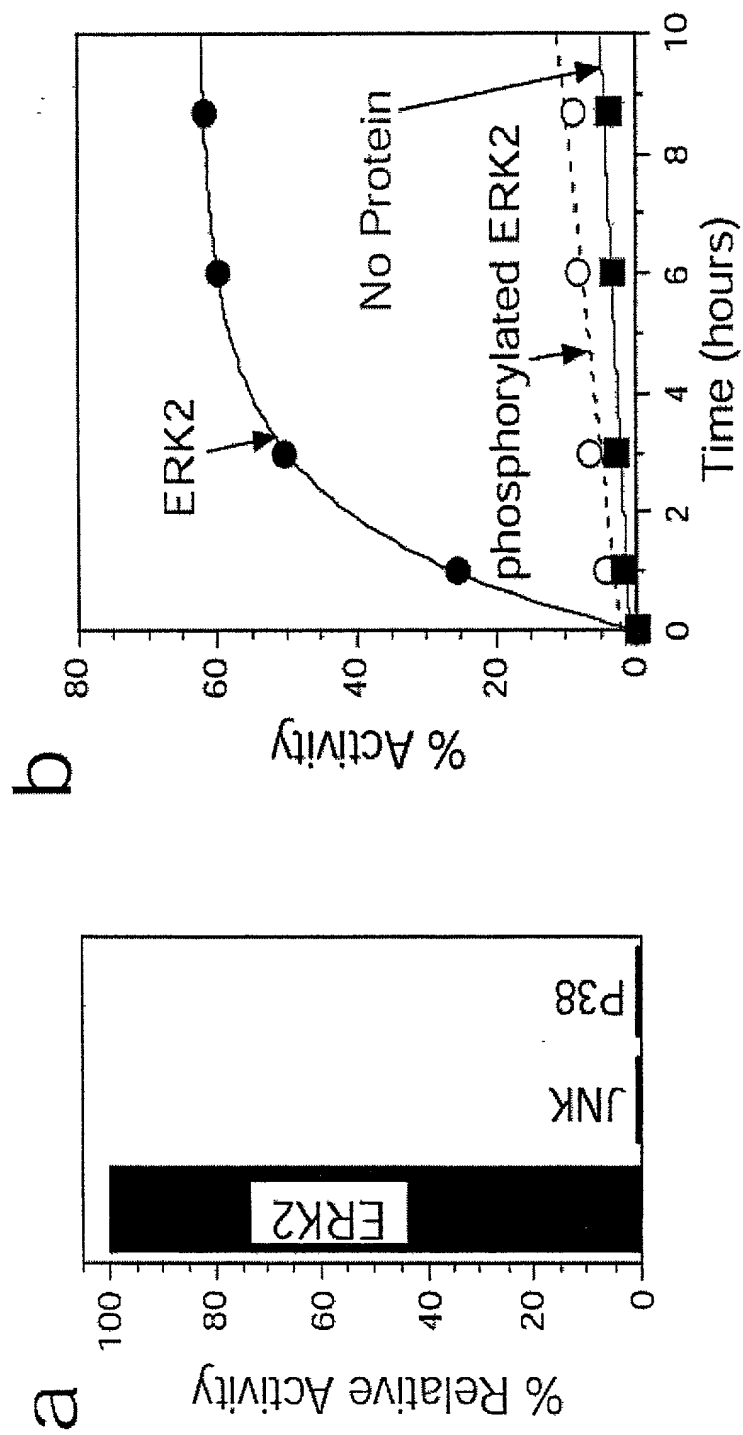


Figure 33: Halfzyme Ligase

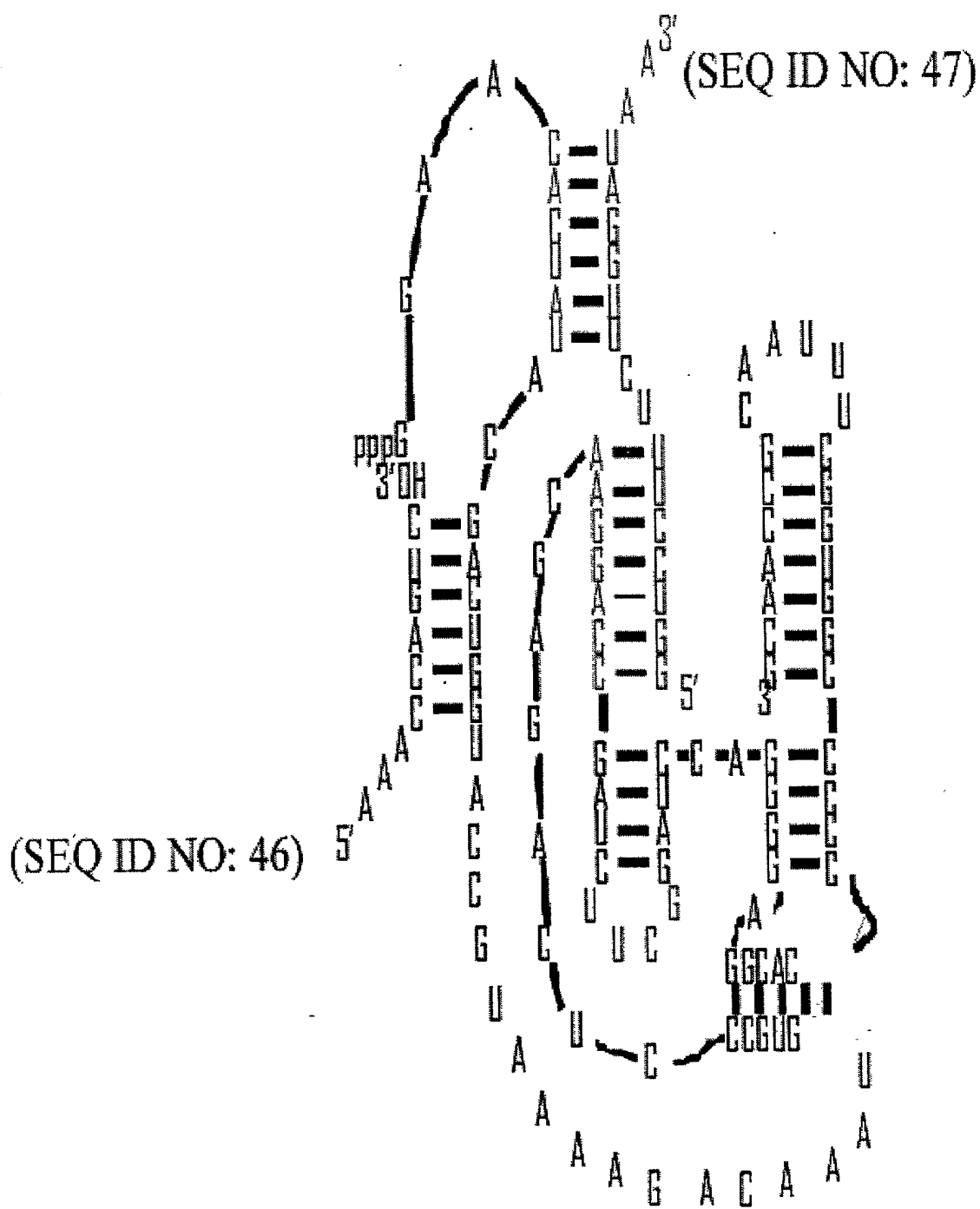


Figure 34: Secondary structure of HCV 5'-UTR

